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Running Head: Apnea Induced Arrhythmia at Altitude

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NEW AND NOTEWORTHY

The peripheral chemoreflex increases both parasympathetic and sympathetic drive under chronic hypoxia. We found that this evoked brady-arrhythmias when combined with apneic periods in Lowlanders at altitude, which become relieved through supplemental oxygen. In contrast high altitude residents (Nepalese Sherpa) do not exhibit brady-arrhythmias during apnea at altitude through potential cardio-protective adaptations. The degree of bradycardia and brady-arrhythmias was related to the hypoxic ventilatory response, demonstrating that the chemoreflex plays an important role in these findings.

ABSTRACT

Peripheral chemoreflex mediated increases in both parasympathetic and sympathetic drive under chronic hypoxia may evoke brady-arrhythmias during apneic periods. We determined if: a) voluntary apnea unmasks arrhythmia at low (344m) and high (5050m) altitude, b) if high altitude natives (Nepalese Sherpa) exhibit similar cardiovagal responses at altitude; and c) if brady-arrhythmias at altitude are partially chemoreflex mediated. Participants were grouped as Lowlanders (n=14; age=27±6yrs) and Nepalese Sherpa (n=8; age=32±11yrs). Lowlanders were assessed at 344m and 5050m while Sherpas were assessed at 5050m. Heart rate (HR) and rhythm (Lead-II ECG) were recorded during rest and voluntary end-expiratory apnea. Peripheral chemoreflex contributions were assessed in Lowlanders (n=7) at altitude after 100% oxygen. Lowlanders had higher resting HR at altitude (70±15 vs. 61±15 bpm;$P<0.01$) that was similar to Sherpas (71±5 bpm;$P=0.94$). High-altitude apnea caused arrhythmias in 11 of 14 Lowlanders (junctional rhythm (n=4), 3° atrio-venticular block (n=3), sinus pause (n=4)) not present at low altitude and larger marked bradycardia (nadir -39±18 bpm; $P<0.001$). Sherpas exhibited a reduced bradycardia response during apnea compared to Lowlanders ($P<0.001$) and did not develop arrhythmias. Hyperoxia blunted bradycardia (nadir -10 ±14bpm; $P<0.001$ compared to hypoxic state) and reduced arrhythmia incidence (3 of 7 Lowlanders). Degree of bradycardia was significantly related to hypoxic ventilatory response (HVR) at altitude and predictive of arrhythmias ($P<0.05$). Our data demonstrates apnea-induced brady-arrhythmias in Lowlanders at altitude but not in Sherpa (potentially through cardio-protective phenotypes). The chemoreflex is an important mechanism in genesis of brady-arrhythmias and the HVR may be predictive for identifying individual susceptibility to events at altitude.

Key Words: Hypoxia; Arrhythmia; Apnea; Sherpa; Chemoreflex

INTRODUCTION

It has been traditionally shown that efferent sympathetic and vagal outflow to the heart is reciprocal, through which increased activation of one pathway sees a respective decrease in the other (17). As such, the variation of cardiac sinus conduction is controlled through the balance between neural outflows in healthy populations. Concurrent increases in both pathways can occur under specific circumstances, and has previously referred to as cardiac "autonomic conflict" (29). This has previously been previously reported during periods of considerable autonomic stress (e.g. cold water submersion) due to conflicting activation of both sympathetic and parasympathetic pathways, ultimately promoting cardiac arrhythmogenesis in healthy individuals (8, 33). In addition, conflict can be seen to some degree in clinical populations suffering from sleep apnea, where elevated chemoreflex gain during apneic periods being is linked to both hypertensive and bradycardia responses (24, 30). Yet the degree of conflict can be considered minimal, with no previous accounts of arrhythmogenesis being noted in healthy populations exhibiting normal chemoreflex function during apnea (e.g. volitional breath holding).

Heightened chemoreflex activity under chronic hypoxia results in a concurrent increase of efferent peripheral sympathetic nerve activity and cardiac vagal tone (9). However these increases are normally dampened by the inhibitor influence of pulmonary stretch receptors, ultimately blunting sympathetic outflow to heightened chemoreflex stress (19, 28, 31). As such, there is limited electrocardiographic evidence that suggests chronic hypoxia exposure leads to incidences of bradycardic arrhythmia within healthy individuals during sleep (4). The peripheral chemoreflex has been implicated specifically in these cases as the changes in heart rate observed appear correlated to the ventilatory response to acute hypoxia (22). Thus, there is a mechanistic basis for hypothesizing that chemoreflex sensitization during acclimatization at altitude (10) may promote autonomic conflict and potential bradycardic arrhythmic events during periods of sleep-related apnea. The use of voluntary apnea during chronic altitude exposure is therefore a relevant experimental model to investigate autonomic cardiac function independent of underlying comorbidities.

Recently, we conducted a high altitude research expedition to 5050m in the Himalayan mountain range of Nepal. Our goal was to study autonomic function during chronic hypoxic exposure in Lowlanders. We contrasted these data with high altitude natives (Nepali Sherpa) to examine whether they would have similar vagal drive as Lowlanders, despite generations of residency at altitude. Our study was designed to experimentally investigate a) if voluntary apnea would unmask vagal mediated bradycardia or conduction abnormalities during wakefulness at altitude (5050m), b) the degree to which the peripheral chemoreflex plays a role in the susceptibility to bradycardic arrhythmia at altitude and c) if low and high altitude natives exhibit similar cardiovagal responses to apnea. Based on previous findings we hypothesized that voluntary apnea in the awake state would experimentally unmask heightened vagal activity and indications of autonomic conflict in Lowlanders at altitude, characterized by bradycardia and arrhythmias.

METHODS

Study Participants

Fourteen Lowlanders (2 female; age=27±6yrs) and 8 highland Nepalese Sherpas (age=32±11yrs) from the Khumbu region of Nepal participated after providing informed written consent. All procedures were explained in English and Nepalese and approved by the University of Alberta Biomedical Research Ethics Board, the University of British Columbia Clinical Research Ethics Board, and the Nepal Health Research Council (Pro00064195) in compliance with the declaration of Helsinki. Health-history screening was negative for any pre-existing cardiovascular, respiratory or neurological disorders. Four Sherpa were current smokers (0.42±0.7 pack years).

Resting Baseline and Apnea Protocol

Pre-expedition testing (Lowlanders) was conducted at 344m (Kelowna, Canada). After flying to 2840m participants followed a conservative ascent profile (9-10 days) to the EV-K2-CNR research facility (5050m; Nepal). Two Lowlanders were administered medication for treatment of altitude illness during ascent (a single dose of acetazolamide (125mg) or dexamethasone), but were tested following a minimum 48hr washout period. Sherpa were tested on days 1-4 and Lowlanders were tested after 4-10 days at 5050m. Both Sherpa and Lowlanders exhibited similar resting peripheral oxygen saturation ($SpO_{2,}$ 82% and 83% respectively) at 5050m.

Participants were tested in the supine position. ECG (Lead II) and arterial blood pressure (finger photoplethysmography; Finometer Pro, Finapres Medical Systems, Netherlands) were collected continuously at 1KHz (ADInstruments, Chart Pro v8.3.1). Brachial arterial pressure waveform was back calibrated through return to flow (RTF) correction confirmed against manual brachial measurements. Mean (MAP), systolic (SBP) and diastolic (DBP) pressures were calculated on a beat-by-beat basis from the calibrated pressure waveform. Beat-by-beat cardiac output (CO) was calculated using the Model Flow algorithm and used to calculate total peripheral resistance (TPR = MAP/CO). SPO_2 was continually assessed (pulse oximetry; Nellcor, Medtronics,USA). Following 10 minutes of quiet baseline measures participants were instructed to perform an end-expiratory apnea (at functional residual capacity). An investigator paced participants' breathing and signaled when to initiate apnea. Participants wore a nose clip and were instructed to "hold their breath for as long as possible". The role of the peripheral chemoreceptors was assessed in seven Lowlanders by repeating apnea at altitude after 1-2 min of pre-breathing 100% oxygen. In addition, individual hypoxic ventilatory responses (HVR; ΔVentilation/ΔSpO_2) at altitude were recorded in a subset of Lowlanders within our study (n=11; 1 female) and Sherpa (n=6) by breathing a fixed FiO_2 (~16%) for five minutes while at 5050m. However, this HVR response was only measured once and not during successive periods while at altitude. We were unable to obtain the HVR response in three of the fourteen Lowlanders and two of the eight Sherpa. These HVR measures were performed independent of the study, though they were at similar time points.

Data and Statistical Analysis

In order to determine if voluntary apnea unmasks autonomic conflict in Lowlanders and Sherpa at altitude; values were calculated at two periods within each group (Lowlanders, lowlanders with supplemental oxygen, and Sherpa) and condition (low and high altitude). Baseline values were calculated over 5 minutes of spontaneous breathing. Electrophysiological characteristics (waveform amplitudes, durations and intervals) of the ECG were assessed during the 30 sec immediately preceding apneas; cardiac cycles (15-45) were over-laid, aligned with the R-wave and the aggregate was analyzed using automated software (Chart Pro 8.3.1). To account for variation in apnea duration, cardiovascular data from the final 10 cardiac cycles of each apnea were analyzed. A cardiologist (SVD) identified and classified conduction abnormalities from ECG waveforms from the 3 beats immediately preceding and 3 beats following apnea breakpoint.

Baseline heart rate variability (HRV) was calculated during 5 minutes to contrast the relative contribution in sympathetic and parasympathetic activation between low and high altitude, and under supplemental oxygen at high altitude. HRV was analyzed using commercially available software (ADI, MLS310/8 HRV, Colorado Springs, CO, USA). Frequency domain analyses included spectrum analysis of very low (VLF; 0-0.04 Hz), low (LF; 0.04-0.15 Hz), and high (HF; 0.15-0.40 Hz) frequency bands. Temporal domain analyses included the standard deviations of the deviations between successive RR intervals (SDNN) and Root Mean Square of the Successive Differences (RMSSD). Total power was calculated as the variance of all NN intervals. The ratio of LF to HF power (LF/HF) was also used.

Statistical analyses was performed using Sigma Stat 3.13 (Systat Software, Chicago, IL). Results are reported as mean ± standard deviation. Differences in cardiovascular data between conditions (low vs. high vs. high+ oxygen) and between groups (Lowlanders vs. Sherpa) were assessed using pre-planned contrasts (paired and unpaired T-tests). Mann-Whiney tests were run in incidences of non-normal distributions. Differences in incidence of arrhythmias between conditions in Lowlanders were assessed using McNemar's test for paired dichotomous data. In order to correct for multiple comparisons (c), the *a priori* alpha (α, 0.05) was adjusted (α') using the experiment-wise error rate (α_e) (15, 32):

$$\alpha' = \frac{\alpha_e}{c}$$

$$\alpha_e = 1 - (1 - \alpha)^c$$

Relationships between measures were assessed using Pearson correlations. Receiver operating characteristic (ROC) curve analysis was performed to assess the specificity and sensitivity of an individual's HVR to predict the susceptibility to arrhythmia during apnea at altitude.

RESULTS

All 14 Lowlanders were successfully tested at 344m and 5050m. No sex differences were present between the 2 females in the Lowlander group. Prior to testing one Lowlander was categorized as having mild acute mountain sickness at altitude (Lake Louise score 3), but no other participants exhibited symptoms of illness.

Group characteristics and cardiovascular function for each condition are reported in Table 1. HR increased in Lowlanders at high altitude ($p<0.05$ vs. low altitude); becoming similar to Sherpas. SBP, DBP, MAP, CO, and TPR were unchanged in Lowlanders at high altitude (SBP P= 0.060; DBP P= 0.782; MAP P=0.901; CO P=0.159 and; TPR P=0.056) and no different than Sherpas (SBP P= 0.786; DBP P=

0.287; MAP P=0.641; CO P=0.581 and; TPR P=0.789). High altitude HVR was not different between Lowlanders (1.11± 1.78 L/min/% desaturation) and Sherpa (0.28± 0.16 L/min/ % desaturation; P=0.317). This remained non-significant even after accounting for one apparent Lowlander "outlier" with a high HVR (6.2 L/min/ % desaturation; P=0.242).

Ascent to altitude saw a shortening of the P-R interval (P<0.001) and P-wave duration (P<0.05), QRS complex widening (P<0.001), QTc prolongation (P<0.001) and P-wave amplitude depression (P<0.001) in Lowlanders (Table 2). Sherpas also exhibited widened QRS complexes (P<0.001) and longer QTc (P<0.05) at altitude compared to Lowlanders at low altitude (Table 2). The incidence of arrhythmia at rest was low in all groups and conditions (Table 3). One Lowlander exhibited periodic premature ventricular contractions during rest at both altitudes, but the incidence remained low (<2/min) and unchanged between altitudes. Upon accent to altitude one Lowlander developed persistent junctional rhythm that was not relieved with oxygen. No arrhythmias were noted in Sherpa at rest.

Lowlanders saw no difference in indices of HRV (SDNN, RMSDD, and total power) between low and high altitude with exception to RRI, which became significantly decreased (1157± 240ms versus 979±208 ms; P<0.001) at high altitude. However, oxygen supplementation did not relieve SDNN, RMSDD, total power, and RRI at altitude. Sherpas did not exhibit any difference in HRV measures to Lowlanders at high altitude. For all groups/altitude there was no difference in frequencies (VLF, LF, and HV) and total power. However, Lowlanders exhibited a significant increase in the LF/HF ratio at high altitude (P<0.05), while both the supplemental oxygen group and Sherpas LF/HF ratio was not different at altitude.

Responses to Voluntary Apnea at Low Altitude

At low altitude, Lowlander SpO_2 was 98±1% prior to apneas but was not obtained during or immediately post-apnea. Lowlanders had an apnea duration of 30.4± 11.1s (range 15-74s) which resulted in a modest bradycardia response (-10±15 bpm; $P<0.001$) (Figure 1). No changes in ECG parameters were noted during apnea at low altitude, although 3 of the 14 Lowlanders developed arrhythmia (premature atrial contractions, atrial bigeminy, and non-conducted sinus beat; Table 3).

Responses to Voluntary Apnea at High Altitude

At high altitude, resting SpO_2 was similar in Lowlanders (82±3%) and Sherpa (83±4%; $P=0.933$). Apnea resulted in further desaturation of Lowlanders (nadir 78±7%) and Sherpa (nadir of 75±5%; $P=0.344$ versus Lowlanders at altitude). Apnea duration was also reduced in Lowlanders [15.4±5.3 s (range 9-27s), $P<0.001$ compared to low altitude] that was similar to Sherpa (15.8±2.6; Range 12-19s).

Lowlander apneas saw magnified bradycardia at high altitude (-39± 18 bpm; $P<0.001$ versus low altitude). In contrast, Sherpa had a reduced extent of bradycardia during apnea (-7± 10 bpm; $P<0.001$ versus Lowlanders). Despite bradycardia blood pressure progressively rose in Lowlanders to a peak MAP at low altitude (112± 19mmHg; $P<0.001$ versus baseline) and high altitude (100±21 mmHg $P< 0.01$ versus baseline). Peak blood pressure response was similar in Sherpa (100± 12 mmHg; $P=1.035$ versus Lowlanders at altitude) (Figure 1). Between rest and apnea only QTc duration was significantly reduced ($P<0.01$) in Lowlanders. Apneas at high altitude resulted in a prolongation of the QRS ($P<0.01$) and PR intervals ($P<0.05$) while P and T wave amplitudes were depressed ($P<0.001$) in Lowlanders. Sherpas and Lowlanders exhibited mostly similar ECG values during apnea at altitude. However, Sherpa P-wave duration was significantly prolonged ($P<0.05$) compared to Lowlanders.

At high altitude there was a greater incidence in arrhythmias during apnea in Lowlanders (11 of 14; $P<0.05$) compared to low altitude apnea. Identified arrhythmias included sinus pause/arrest, junctional, and 3° atrio-venticular block (Figure 2; Table 3). In contrast, no abnormalities were apparent during apnea in Sherpas, despite no differences in the duration of apnea or degree of desaturation when compared with lowlanders at altitude.

As HVR was similar between groups (see above), including the one female participant, data were combined to assess the relationship between HVR and heart rhythm. The gain of the bradycardic response during apnea across groups was correlated with HVR (Figure 3). Two Lowlanders were identified as statistical outliers using studentized residuals; however, the relationship remained significant when these individuals were either included or removed from the analysis (Figure 3). When data were grouped based on the presence or absence of arrhythmia during apnea, those individuals exhibiting apneas had significantly higher HVRs (Median 0.66 L/min) vs. those that did not exhibit arrhythmias (Median 0.26, $P<0.02$; Figure 4a). ROC analysis further indicated that HVR was significantly predictive of the incidence of arrhythmia during apnea (AUC = 0.86, $P<0.05$; Figure 4b) with a sensitivity of 75% and specificity of 78% when using an optimized HVR cutoff of 0.40 L/min (Figure 4c). However, there was no relationship between HVR and the magnitude of bradycardia during apnea (R^2= 0.08).

Influence of Supplemental Oxygen to Apneic Response at High Altitude

Supplemental oxygen was administered to 7 out of the 14 Lowlanders at high altitude prior to voluntary apnea. This increased initial SpO_2 from 82± 3 % to 96± 1 % ($P<0.001$) prior to apnea and reduced resting heart rate (62± 10 bpm; $P<0.05$ versus altitude). Apnea duration was prolonged (67.0± 45.2 s; $P<0.01$ versus euoxic apnea) following oxygenation. Oxygenation significantly blunted apnea related bradycardia (Figure 1; $P<0.05$) but increased associated peak in MAP (117± 16mmHg; $P<0.05$). Supplementation of oxygen returned R-wave amplitude to low altitude values and the incidence of arrhythmia was significantly reduced compared to euoxic apnea (3 of 7 Lowlanders, $p<0.05$; Table 3).

DISCUSSION

In the current study we have demonstrated that through the use of voluntary apnea at altitude, there exists considerable underlying vagal and sympathetic drive in Lowlanders as marked by significant bradycardia and incidence of brady-arrhythmias. In contrast, Sherpas exhibited a less pronounced bradycardia during apnea and an absence of arrhythmias. Using supplemental oxygen we further demonstrated that the augmented bradycardia and arrhythmias observed in Lowlanders were specifically related to the peripheral chemoreflex. This was also supported by a significant relationship between the participant specific HVR and the degree of bradycardia occurring during apnea. Furthermore, ROC analysis indicated that heightened HVR was significantly predictive of the susceptibility to high altitude arrhythmias during apnea.

At altitude Lowlanders exhibited shorter P wave duration and PR Interval; as well as enlarged P and R wave amplitudes, suggesting an elevated sympathetic drive as seen previously (11, 14). In clinical contexts shortening of P and PR intervals is associated with increased risk of atrial fibrillation (1, 26). Despite changes in ECG conductance, arrhythmias during baseline were not observed in either Lowlanders or Sherpa. Previous accounts altitude related arrhythmias in Lowlanders have been reported. These have been primarily documented during periods of physical exertion after rapid accent (3) or during sleep (4). Similar to our findings, these events were also associated with flattened T-wave amplitudes and p-wave shortening(3). Recently, Woods *et al.* (34) noted the presence of symptomatic sinus tachycardia at altitude (n=2) during periods of strenuous exercise via implantable loop recorder; where Brooks *et al.* (4) also noted arrhythmias during periods of exertion periods at altitude in 16 Lowlanders using continuous ECG monitoring. In both reports, arrhythmia incidence was increased at higher altitudes (5000-7550m) as well as with longer exposure periods (4, 34). Both our data and these previous findings suggest high altitude to be a “pro-arrhythmia” environment, where the influence of hypoxia may be compounded further during periods of stress. Although we did not obtain continuous

ECG monitoring through the study; our findings agree with the presence of arrhythmias during periods of heightened stress.

Previous studies show increased periodic breathing and central apnea at altitude in Lowlanders (2, 5, 27); as well as associated periods of bradycardia (16, 20, 22, 27) and arrhythmia (6, 16, 20). Thus, the current study utilized voluntary apnea to characterize the mechanisms of altitude related bradycardia and arrhythmia in Lowlanders at rest. The significant bradycardia as well as the development of arrhythmias (11 of 14 Lowlanders) during apnea is indicative of heightened sympathetic and parasympathetic innervation of the heart at altitude (9, 17, 25). Although the relationship sympathetic and parasympathetic control is often considered reciprocal, when both innervations are concurrently elevated the heart experiences what has previously been termed as "autonomic conflict". The occurrence of cardiac arrhythmias during cold-water immersion has been attributed to this conflict (29) when high sympathetic (cold shock response) and parasympathetic (mammalian diving reflex) activity occurs. As classically described, the primary cardiovascular consequences of peripheral chemoreceptor activation are concurrently elevated sympathetic and parasympathetic activity (9). Yet under eupneic conditions hypoxia engages pulmonary reflexes (via the hypoxic ventilatory response) that inhibit both parasympathetic (9) and sympathetic activity (19, 28, 31). The degree of chemoreflex sensitivity and its direct relationship to sympathetic augmentation at altitude is not fully understood. Despite this, earlier works have demonstrated that the augmentation of sympathetic activity under acute hypoxia exposure is driven through heightened peripheral chemoreflex activation (23, 35). In the current study we have demonstrated a similar "autonomic conflict" that is unmasked during apnea and mediated via the peripheral chemoreflex. Firstly, we showed that the bradycardia associated with apnea at altitude was correlated with the hypoxic ventilatory response. Secondly, oxygen administration prior to apnea at altitude eliminated bradycardia and reduced the incidence of arrhythmia to the same level as observed at low altitude.

Although Sherpas had a similar breath hold duration and resting arterial oxygen saturation; they did not exhibit significant bradycardia or any arrhythmias. Previous data from native Tibetans indicate a normal ECG compared Han residents who had migrated during childhood to high altitude (13). We saw that breath holding generated arrhythmias in most Lowlanders but not Sherpa. Thus, these findings suggest that Sherpa exhibit an altered cardiac response to hypoxic stress. However, it is unclear what the specific nature of the adaptation that might be present. We do not believe this is related to differences in chemoreflex sensitivity as our findings are consistent with recent data indicating Sherpas to have similar chemoreflex sensitivity to acclimatized Lowlanders (7, 12, 36). Yet when Lowlanders and Sherpa were considered together, we found that high altitude HVR was significantly related to the normalized bradycardic response to high altitude apnea.   Previously, Masuyama *et al.* (21) observed a significant relationship between low altitude HVR and high altitude sleep related (normalized) bradycardia. These previous data are intriguing as the relationship they demonstrate is apparent across conditions (low vs. high altitude) and sleep state (awake vs. sleep). This would suggest a robust predictive utility of low altitude HVR. In keeping with this, we demonstrated that high altitude HVR was significantly predictive of arrhythmias during apnea at altitude and therefore potentially useful as a predictor for risk of high altitude arrhythmia.

Considerations

The current study was apart of a larger research expedition to the Himalayan Range in Nepal and involved several independent studies examining vascular, cerebral auto-regulatory, neuromuscular, and autonomic function between Lowlanders and Sherpa. As such, certain time-dependent and technical limitations exist with regards to testing both Lowlanders and Sherpa at altitude. One limitation was the inability to repeat the supplemental oxygen trial within Sherpa. Sherpa were initially tested at 5050m and soon followed by Lowlanders. Yet significant arrhythmic events were only noted in Lowlanders at 5050m. Following several examples of arrhythmias within Lowlanders we attempted to address the potential chemoreflex contribution though supplemental oxygen. However, Sherpa exhibited neither considerable bradycardic responses nor arrhythmias during apnea. Therefore, we believe that supplemental oxygen would not have produced any considerable difference in cardiovagal responses during apnea.

We demonstrate a relationship between the HVR and degree of bradycardic response at altitude. However, we acknowledge that two individuals with high HVRs (statistically identified as outliers in relation to the rest of our participants) appear to weight this relationship. However, even if these two participants were removed, there still existed a significant relationship between HVR and bradycardic response (see figure 3). We acknowledge that our measure of HVR may exhibit some degree of ventilatory suppression through respiratory induced alkalosis, thus further minimizing central chemoreceptor activation. This limitation within our HVR measure should be considered during interpretation of results. However, our specific goal was to assess peripheral chemoreceptor contributions within a high altitude field setting. Due to the nature of the technique that was utilized for determining HVR (measuring the ventilatory response to continuous 16% FiO_2 at 5050m) without successive measurements; it would be recommended that utilizing HVR for predicting bradycardic events should be investigated further to confirm the present findings.

Conclusion

Our results suggest increased parasympathetic activity and sympathetic drive at altitude promote "autonomic conflict" during apnea. Thus, potential conflict may promote both cardiac changes and arrhythmia development in Lowlanders that travel to higher elevations for work or pleasure. Since high altitude HVR appears to be predictive of arrhythmia incidence; further evaluation of low altitude HVR should be evaluated for predicting the susceptibility of to high altitude brady-arrhythmia. The lack of arrhythmias in Sherpa suggests an adaptive mechanism, though it is unclear if the mechanism behind this response is due to generational adaptation.

AUTHOR CONTRIBUTIONS

The experiments within this study were conducted at both the Center for Heart, Lung, and Vascular Health (UBC Okanagan, Kelowna; Canada) and the EV-K2-CNR Research Facility (Lobuche; Nepal). Co-authors listed have contributed to either i.) conception or design of work (CDS, MS, JPM, and SAB), ii.) acquisition, analysis, or interpretation of data for the work (SAB, HD, FS, LR, LS, CDS, JPM, MS, and SVD), or iii.) drafting the work or revising it critically for important intellectual content (PNA, CKW, SAB, CDS, RH, JPM, and MS). All persons listed have qualified for authorship and approve of the final version of the manuscript. Finally, all authors listed agree to being accountable with regards to ensuring accuracy and integrity for the work currently investigated.

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DISCLOSURES

The authors declare no conflicts of interest, financial or otherwise.

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FIGURE LEGENDS

FIGURE 1. Responses to apnea in Lowlanders at 344m (n.14; black circle), Lowlanders at 5050m (n.14; white circle), Lowlanders + O2 at 5050m (n.7; blue circle), and Sherpa at 5050m (n.8; red circle). *Panel A,* Absolute bradycardia response to apnea. *Panel B,* Percentage change of bradycardic response to apnea. A significant bradycardia response was observed in Lowlanders at high (but not) low altitude. Apnea after 100% oxygen eliminated bradycardia in Lowlanders. Sherpa did not exhibit bradycardia during apnea at altitude. *Panel C,* Absolute pressor response during apnea. *Panel D*, Percentage change of pressor response during apnea. All data has been aligned to break-point and the last 10 cardiac cycles have been plotted. The mean nadir/peak responses are also identified. *Lowlanders at high altitude significantly different from all other groups, $P < 0.05$; † Significant difference between Lowlanders at low altitude vs. Lowlanders at high attitude, $P<0.05$; ‡ Significant difference between Lowlanders at low altitude and Sherpa, $P<0.05$; **§** Significant difference between Lowlanders + Oxygen (n.7) at altitude and Sherpa, $P<0.05$; ‖ Significant difference between Lowlanders + Oxygen and Lowlanders (without oxygen) at altitude, $P<0.05$.

FIGURE 2: Raw data demonstrating apnea induced arrhythmia at altitude. Examples of ECG tracings from the same male participant during apnea at low (top) and high altitudes (bottom). Apnea at altitude exhibited arrhythmic events, such as 3° heart block (see inset bottom right)

FIGURE 3: Correlation analysis between the normalized bradycardic response to apnea and hypoxic ventilatory response at altitude across groups. Closed circles represent Lowlanders (n.14) and open circles represent Sherpa (n.8). Two lowlanders were identified at statistical “outliers” (red symbols) based on studentized residuals. However, a significant relationship was also maintained if these participants were excluded (inset). The dashed lines and dotted lines represent the linear regressions and 95% confidence intervals respectively.

FIGURE 4: Hypoxic ventilatory response was higher in individuals who developed arrhythmias during apnea (**A**). Receiver operating curve analysis indicated that the hypoxic ventilatory response was significantly predictive of the incidence of arrhythmia at altitude (**B**). When an optimal cut-off was determined (0.40 L/min/% desaturation), the hypoxic ventilatory response was predictive of arrhythmias with a sensitivity of 75% and specificity of 78%.

TEXT TABLES

Table 1: Demographic, cardiovascular, and sympathetic function in Lowlanders and Sherpa at low and high altitudes.

	LOWLANDERS			**SHERPA**
	334m (n =14)	5050m (n =14)	5050m +Oxygen (n =7)	5050m (n =8)
Subject Demographics				
Age (years)	27± 6	27± 6	30± 8	32± 13
Height (m)	1.77± 0.8	1.77± 0.8	1.79± 0.06	1.68± 0.08
Weight (kg)	72.2± 10.1	69.4± 8.6	69.3± 10.3	63.7± 10.1
BMI (kg/m^2)	23.1± 2.8	22.2± 2.5	21.5± 2.9	22.8± 3.5
Resting Cardiovascular Function				
Heart Rate (bpm)	61± 15	70± 15*	62± 10*	71± 5‡
SPO_2 (%)	98± 1	82± 3	96± 1 †	83± 4
Systolic Pressure (mmHg)	119± 9	113± 13	113± 8	111± 9
Diastolic Pressure (mmHg)	66± 7	70± 10	71± 8	65± 8
Mean Pressure (mmHg)	84± 8	86± 11	89± 7	84± 9
Cardiac Output (L/min) ♦	5.9± 1.8	5.5± 1.4	5.1± 1.1	6.0± 1.7
Total Peripheral Resistance♦	15± 4	17± 4	19± 7	16± 7

♦ Values calculated using Model Flow.
* Significantly different from Lowlanders tested at low altitude (334m); $p < 0.05$.
† Significantly different from Lowlanders tested at high altitude (5050m); $p<0.05$.
‡Significantly different from Lowlanders during hyperoxia (5050m + Oxygen); $p<0.05$.

Table 2: Electrocardiogram measurements made in Lowlanders and Sherpa at low and high altitudes during rest and apnea.

	LOWLANDERS			**SHERPA**
	334m (n =14)	5050m (n =14)	5050m +Oxygen (n =7)	5050m (n =8)
REST				
P-wave duration (ms)	96± 10	77± 20*	87± 12	97± 16†
P-wave amplitude (mV)	0.16± 0.05	0.14± 0.04	0.14± 0.06	0.11± 0.02*‡
PR-Interval (ms)	169± 19	124± 27*	146± 51	158± 38†
QRS duration (ms)	71± 14	121± 3*	119± 6 *	120± 1*
▲QTc (ms)	398± 26	456± 28*	449± 23 *	408± 15†‡
R-wave amplitude (mV)	1.76± 0.70	1.24± 0.60	1.49± 0.45 *	1.19± 0.22*
T-wave amplitude (mV)	0.49± 0.19	0.28± 0.14*	0.39± 0.19 *	0.36± 0.15*
APNEA				
P-wave duration (ms)	96± 20	74± 27	68± 22	68± 34†‡
P-wave amplitude (mV)	0.15± 0.05	0.10± 0.06*	0.10± 0.06*	0.09± 0.06*
PR-Interval (ms)	163± 31	128± 26*	151± 55	157± 37
QRS duration (ms)	74± 13	119± 4*	117± 5*	113± 10*
▲QTc (ms)	398± 34	410 ± 33§	417± 31*	465± 51‡
R-wave amplitude (mV)	1.67± 0.79	1.45± 0.74	1.65± 0.60*	1.25± 0.34
T-wave amplitude (mV)	0.48± 0.19	0.32± 0.18*	0.40± 0.28	0.37± 0.13

All measurements were taken using a standard lead II configuration. Measurements during apnea were taken from the 10 cardiac cycles prior to volitional breakpoint

▲Framingham correction (QT+0.154*(1-RR).

***** Significantly different from Lowlanders tested at low altitude (334m); p <0.05.

† Significantly different from Lowlanders tested at high altitude (5050m); p<0.05.

‡ Significantly different from Lowlanders during hyperoxia (5050m +Oxygen); p<0.05.

§ Significantly different from rest within the same condition/group; p<0.05.

Table 3: ECG conduction abnormalities identified at rest and during voluntary apnea.

	LOWLANDERS			SHERPA
	334m (n =14)	5050m (n =14)	5050m +Oxygen (n =7)	5050m (n =8)
ABNORMALITIES IDENTIFIED AT REST				
Premature Ventricular Contractions (< 2/min)	1	1	1	---
Junctional Rhythm	---	1	1	---
ABNORMALITIES ASSOCIATED WITH APNEA*				
Atrial Bigeminy	---	---	1	---
Premature Atrial Contractions	1	---	---	---
Ectopic Atrial Rhythm	1	---	---	---
Non-conducted Sinus Beat	1	---	---	---
Non-conducted sinus beat / Junctional Escape	---	1	---	---
Sinus Pause/Arrest	---	1	---	---
Sinus Pause/Arrest with Junctional Escape	---	2	---	---
Sinus Pause with Junctional Rhythm	---	1	---	---
Sinus Arrest with Junctional Rhythm	---	3	1	---
3º A-V Block	---	3	1	---

ECG assessment carried out by cardiologist (SVP) who was blinded to group and condition. Premature ventricular contractions were observed at rest in the same individual under all conditions; the rate of occurrence did not change with condition. One individual developed persistent junctional rhythm at altitude, this persisted during the oxygen administration.

* All conduction abnormalities associated with voluntary apnea occurred immediately preceding or following (< 3 beats) break-point.

FIGURES

FIGURE 1.

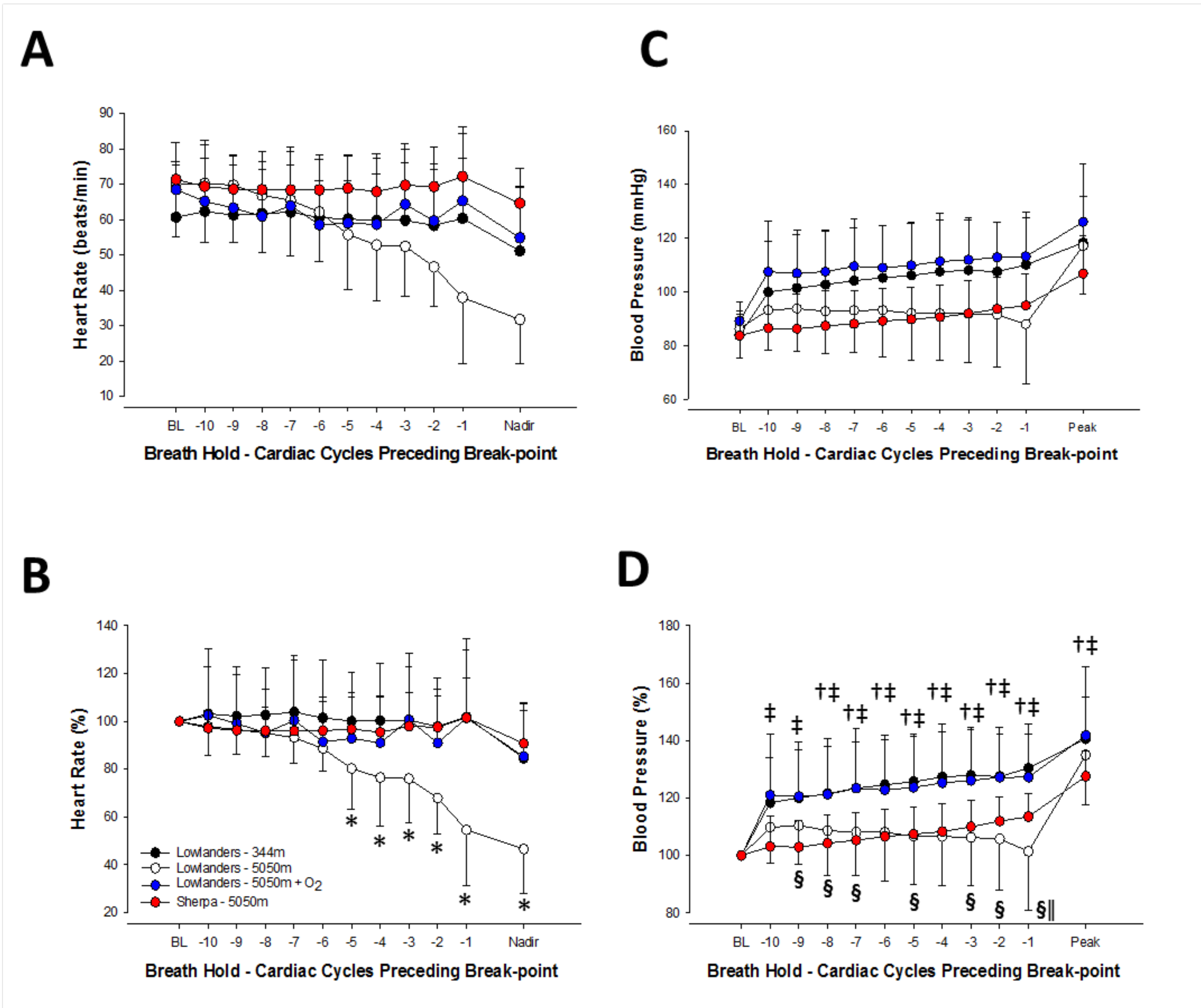


FIGURE 2

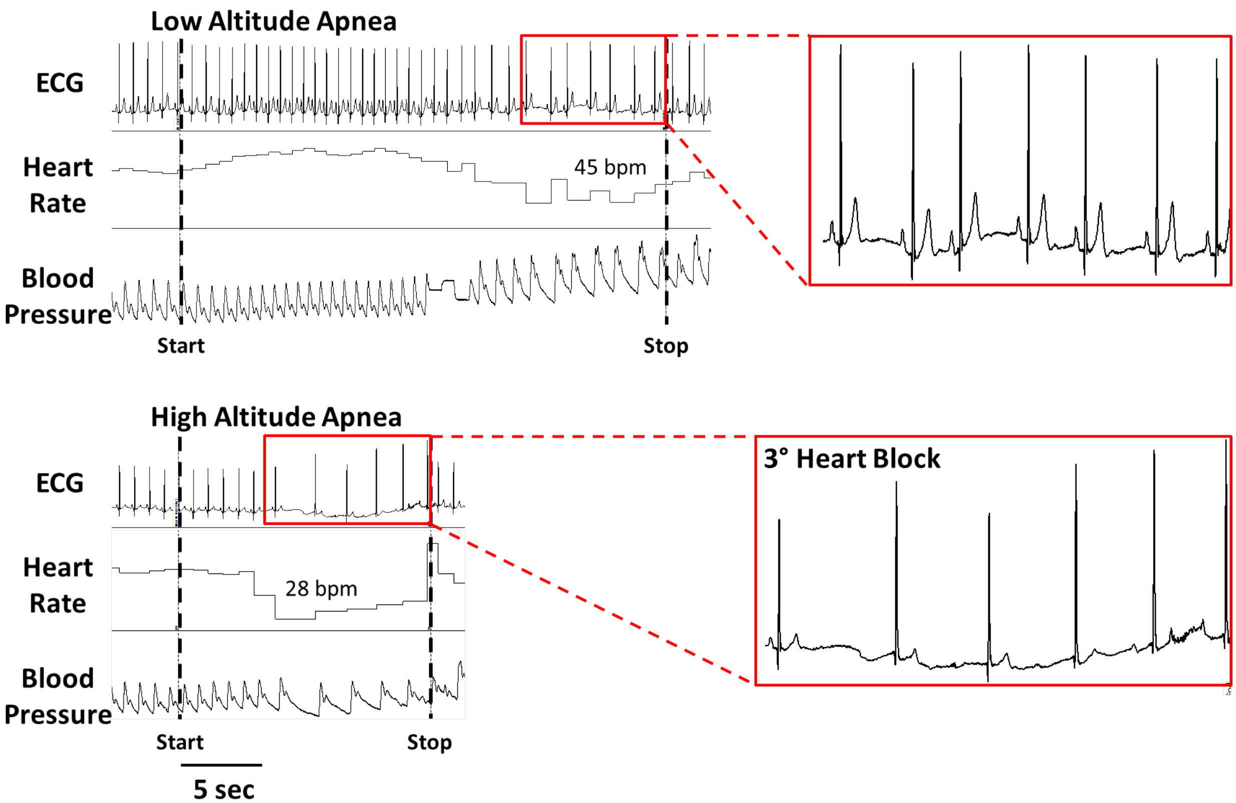
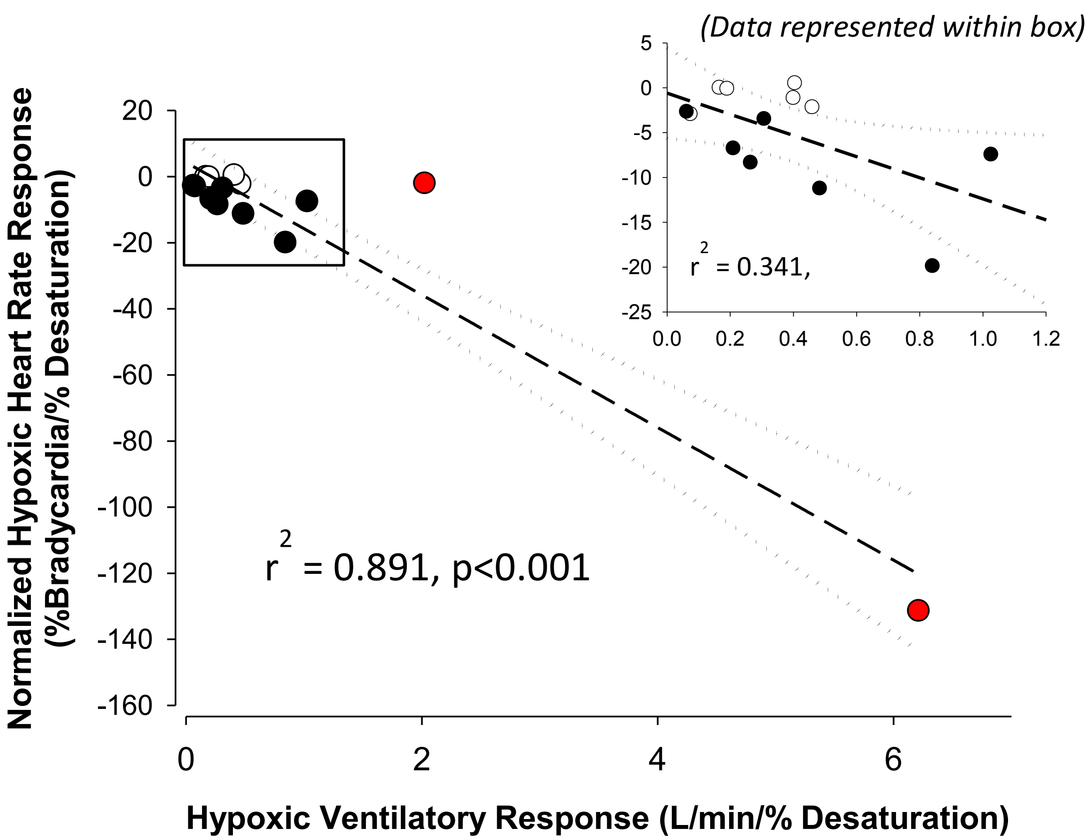
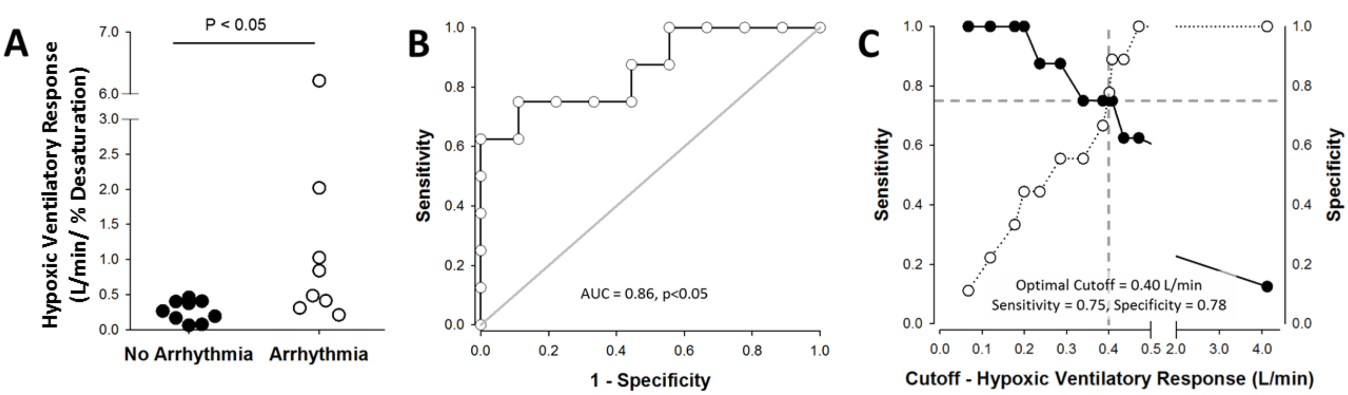

Low Altitude Apnea
ECG
Heart Rate
45 bpm
Blood Pressure
Start
Stop
High Altitude Apnea
3° Heart Block
ECG
Heart Rate
28 bpm
Blood Pressure
Start
Stop
5 sec

FIGURE 3.



(Data represented within box)
Normalized Hypoxic Heart Rate Response
(%Bradycardia/% Desaturation)
Hypoxic Ventilatory Response (L/min/% Desaturation)
r² = 0.891, p<0.001
r² = 0.341,

FIGURE 4


A
P < 0.05
Hypoxic Ventilatory Response (L/min/ % Desaturation)
7.0
6.0
3.0
2.5
2.0
1.5
1.0
0.5
0.0
No Arrhythmia
Arrhythmia
B
Sensitivity
1 - Specificity
AUC = 0.86, p<0.05
C
Sensitivity
Specificity
Optimal Cutoff = 0.40 L/min
Sensitivity = 0.75, Specificity = 0.78
Cutoff - Hypoxic Ventilatory Response (L/min)